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COMPARISON OF THE DEVELOPMENT AND FUNCTION OF THE SWIMBLADDER OF *BREVOORTIA TYRANNUS* (CLUPEIDAE) AND *LEIOSTOMUS XANTHURUS* (SCIAENIDAE)

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Comparison of the Development and Function of the Swimbladder of *Brevoortia tyrannus* (Clupeidae) and *Leiostomus xanthurus* (Sciaenidae)

JOHN J. GOVONI AND DONALD E. HOSS

The swimbladders of physostomous *Brevoortia tyrannus* (Atlantic menhaden) and physoclistous *Leiostomus xanthurus* (spot) develop as a simple evagination of the larval foregut. The swimbladder of *L. xanthurus* develops earlier (at yolk and oil globule depletion when larvae are two days posthatch and ~2 mm notochord length) than it does in *B. tyrannus* (well after yolk and oil depletion when larvae are 12 days and ~8 mm). The larvae of each species initially inflate the swimbladder by ascending to the surface and forcing atmospheric gas through a pneumatic duct into the swimbladder lumen. Larval *B. tyrannus* modulate swimbladder volume and buoyancy within a diel cycle by inflation with atmospheric gas passed through a persistent pneumatic duct and deflation by diffusion through the swimbladder wall along with expulsion through the anus and mouth. This capacity of swimbladder volume modulation and buoyancy compensation corresponds with the average vertical distribution of larvae in the sea, where larval *B. tyrannus* occupy the upper water column. The pneumatic duct of *L. xanthurus* atrophies after 20 days and has no discernable lumen after 40 days, the beginning of metamorphosis when larvae are ~7 mm standard length. The mucosal epithelium of the swimbladder of *L. xanthurus* acquires cytological characteristics of adult gas-gland tissue soon after initial inflation when larvae gain the capability of gas secretion. A plexiform rete mirabile does not form until metamorphosis. The swimbladder of larval *L. xanthurus* functions by providing neutral buoyancy with low amplitude diel depth changes in swimbladder volume. In the sea, larval *L. xanthurus* occupy primarily mid- and deep depths.

THE ontogeny of the teleostean swimbladder has attracted attention since the turn of the 20th century (reviews in McEwen, 1940; Harden Jones and Marshall, 1953; Goodrich, 1958), yet many details of swimbladder derivation and early function remain undescribed and many consequent questions unanswered for many species. In most fishes, the swimbladder develops as a simple out-pocketing of the alimentary canal (e.g., Harden Jones and Marshall, 1953; Goodrich, 1958; Steen, 1970). Although this derivation appears among phylogenetically diverse taxa, the literature suggests the possibility of more complicated derivations. Duwe (1955), and later Schwarz (1971), recognize three modes of swimbladder formation: (1) as an evagination of the gut; (2) as an unconnected mass of mesodermal cells that are later invaded by an evagination of endodermal cells from the gut; and (3) as an independent mass of cells, unidentified as to germ-cell origin, that later sends out a connection to the gut. The first two of these schemes are tenable, but the third gives rise to questions concerning germ cells from which this organ is derived. These questions are closely connected to questions concerning the timing of swimbladder development. Does the swimbladder develop in the embryo where germ cell lines reside (Ballard, 1973a,b) or in the larva where many visceral anlagen and tissues have differentiated?

The timing of swimbladder development also gives rise to questions of tissue renovation. The unsegmented incipient gut of the embryo or yolk-sac larva is lined with mucosal columnar epithelium characterized by an apical striated brush border of microvilli, as is the mid- and hindgut of larvae after yolk and oil-globule depletion, whereas the foregut mucosa is lined with simple cuboidal epithelial cells and mucus cells (Govoni et al., 1986). From what segment of the gut does this evagination arise, in those species with swimbladder anlagen formed from evagination? If the swimbladder forms by evagination of the mid- or hindgut, what sort of tissue differentiation occurs, that is, how does the organ change histologically after its formation? Among physoclistous fishes that develop the swimbladder as an evagination from the gut, when does the connection with the gut, the pneumatic duct, atrophy?

Questions concerning the function of the swimbladder among larval fishes also linger. What is the source of gas for initial swimbladder inflation among species? Does the larval swimbladder secrete gas internally? If the swimbladder looses its open connection with the gut, when does the characteristic gas gland tissue

and rete mirabile develop? How does the swimbladder function for larval fishes in the sea?

Brevoortia tryannus and *Leiostomus xanthurus* are two phylogenetically and physiologically divergent species, a physostomous clupeid and a physoclistous sciaenid. Hoss and Blaxter (1982) describe the gross anatomical development of the swimbladder of *B. tyrannus* in connection with its function, with the inner-ear and lateralis system, in pressure sensation. Hoss and Phonlor (1984) and Hoss et al. (1989) describe larval swimbladder inflation in the laboratory and in the sea. Larval *B. tyrannus* ascend to the surface in the evening and force atmospheric gas into the swimbladder; larvae then sink slowly as gas diffuses through the swimbladder wall into the body tissues (Forward et al., 1993, 1994). Multiple ascensions and reinflations during darkness are possible (Forward et al., 1999) but have not been observed. The development of the swimbladder of *L. xanthurus* is undescribed. In the sea, larvae reside in shallower depths at night than in daylight within an overall range of ± 10 m about middepth (Govoni and Pietrafesa, 1994; J. A. Hare and J. J. Govoni, unpubl.). The detailed development of the larval swimbladder and its function in buoyancy compensation for either species are unknown.

The purpose of this work was to trace the ontogeny of the swimbladder of *B. tyrannus* and *L. xanthurus* (spot); to determine when, and how, the larval swimbladder functions; to predict swimbladder inflation and gas volumes and the vertical distribution of larvae in the sea given the observed buoyancy function of the swimbladder; and to compare inflation and gas volumes and vertical distributions in the sea with these expectations.

Materials and Methods

Morphology.—Larvae were obtained from populations that were spawned and reared in the laboratory (Hettler, 1981; Hettler and Powell, 1981) and from field collections. Larvae were reared in 0.5 m × 0.5 m, 1000-liter cylindrical tanks in 20 ± 1 C, 34 psu seawater at a 12:12 h light:dark cycle (lights on, 0700 h; off, 1900 h). Young larvae (< 20 days) were fed 10 rotifers (*Brachionus plicatilis*) ml^{-1}; older larvae were fed rotifers plus 1 *Artemia nauplius* 2 ml^{-1}.

To ensure that larvae to be studied were well fed, healthy, not of elevated physical density resulting from osmotic stress (Scalfani et al., 1997), and with consistent swimbladder inflation, larvae were selected from near surface of rearing tanks at approximately 0830 h Eastern Standard Time (EST). Age of larvae is reported herein as days after hatching. Lengths are live notochord length before the formation of hypural plates and standard length thereafter (Powell and Gordy, 1980; Hettler, 1984).

Each day, when reared larval *B. tyrannus* were from one to 68 days and larval *L. xanthurus* were one to 48 days, 10 larvae were anesthetized (tricaine methane sulfonate) and five fixed in either 10% phosphate buffered formalin or modified Gendre's fixative (Pearce, 1968). Several larvae fixed in formalin from each age group were prepared histologically with standard techniques for paraffin infiltration and embedding, sectioned at 5 μm, mounted, and stained with Gill's hematoxylin and eosin Y. Larvae fixed in Gendre's fixative were stained with periodic acid-Schiff's reagent (PAS) and counterstained with Gill's hematoxylin. For finer cellular detail, larvae of several ages were embedded in glycol methacrylate, sectioned at 2 or 4 μm, and stained with either alkali blue 6B and neutral red or toluidine blue and acid fuchsin (Govoni, 1980). Juveniles and adults of unknown age from field collections were fixed in 10% borate-buffered formalin in seawater, dissected, and the gross anatomy of the swimbladder examined. Tissue specimens from representative juveniles and adults from field collections were prepared with standard techniques (Govoni, 1980). Terminology of swimbladder anatomy and histology follows Harden Jones (1957). Metamorphosis was defined as the developmental interval between the first appearance of the juvenile form of visceral organs and the adult form; for these species, this typically corresponds with a like transition in external morphology.

Physiology.—To determine the source of gas for initial swimbladder inflation for *L. xanthurus*, larvae that were nearing yolk and oil-globule depletion were placed in three vessels (one, four, and five liters) filled with O_2-saturated seawater that were capped with a 0.5 cm film of mineral oil and sealed with a ground glass stopper to block access to the atmosphere. The concentration of larva was 50 L^{-1} in each vessel. Larvae in these vessels were held with rotifers transferred initially from rearing tanks with larvae, but this food was not replenished. Five larvae were removed from each vessel daily for nine days, the vessels replenished with O_2-saturated seawater, and rapidly resealed. Measurements of swimbladder development and inflation in larvae deprived of access to the atmosphere were compared with those of larvae from reared populations.

To determine when, and with what capacity,

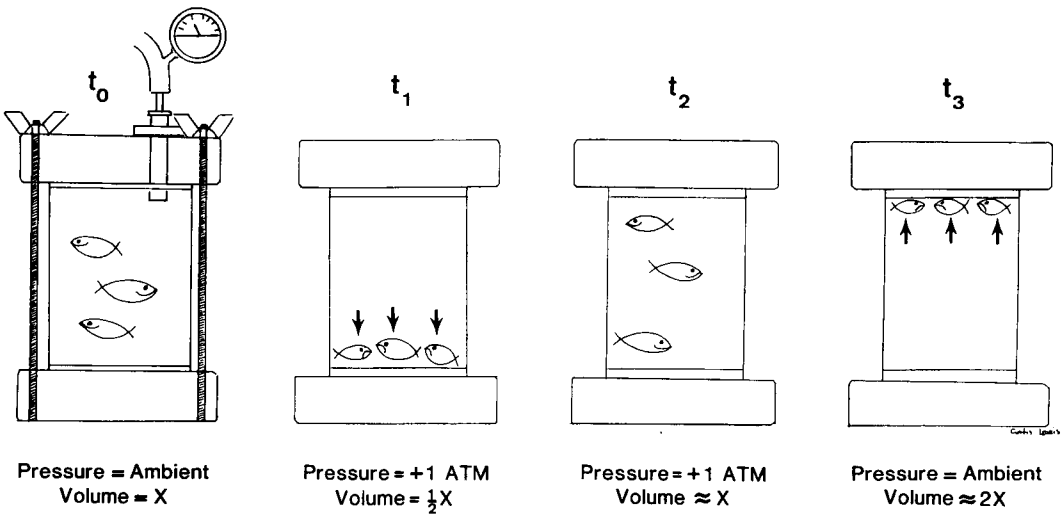

Fig. 1. Cartoon of the gas secretion experiments. (t_0) depicts the position of larval *Leiostomus xanthurus* before application of pressure; (t_1) the position of larvae after initial application 1 atm (101 kPa) of pressure above ambient atmospheric pressure; (t_2) the position of larvae 1 to 7 h after the application of pressure; (t_3) the position at the end of experiments.

larval *L. xanthurus* acquire the ability to secrete gas into the swimbladder, larvae of 13 age groups of from three to 31 days were tested (Fig. 1). Ten larvae were anesthetized and their length and the volume of gas in their swimbladders measured separately before each experiment (because recovery and survival from anesthesia was low). Another 20 larvae were placed in a 4 cm × 6 cm, 70 ml hyperbaric chamber with O_2-saturated seawater and with a 0.5 cm surface layer of mineral oil (to prevent access to any gas bubbles). One atm (101 kPa) above ambient air pressure was applied with compressed O_2. The vertical position of larvae within this chamber was recorded on a video cassette recorder for 7 h; thereafter, the pressure was released and the length of larvae and volume of gas in the swimbladder measured. The recorded position of larvae after the application of gas and before the release of pressure was taken as an indication of gas secretion. The difference between estimates of gas volume of larvae that were measured before hyperbaric treatment and estimates of gas volume of larvae measured after treatment was taken as the volume of gas secreted. Mortality of larvae, resulting from the collision of larvae with the bottom of the hyperbaric chamber with their rapid descent upon pressure application, was high. Gas secretion experiments were conducted in 20 ± 1 C, 34 psu filtered seawater. The temperature of gas measured before, during, and after treatment was 20 ±1 C.

The depth position of larval *B. tyrannus* and *L. xanthurus* taken in the sea was estimated from larvae captured with a multiple opening and closing net and environmental sensing system (MOCNESS; Wiebe et al., 1976) on 1 February 1992, in Onslow Bay, North Carolina (sunrise, 0655 h; sunset, 1734 h EST). Swimbladder volume of larvae taken at depth was measured aboard ship and was adjusted for temperature and pressure change from the depth where they were collected to surface atmospheric conditions with the universal gas law.

The volume of gas in the swimbladder was taken as the volume (V) of a prolate spheroid [$V = 1.333\ \pi\ (0.5a)(0.5b)^2$] with the long (a) and short (b) axes measured with an ocular reticle (Hunter and Sanchez, 1976). The principal source of measurement error was diffusion of gas through the swimbladder wall in the interval between removal of larvae from experimental vessels and collecting devices and the sequential measurement of swimbladder dimensions in individual larvae.

Results

Morphology: Brevoortia tyrannus.—Evagination was first seen in the caudodorsal wall of the foregut at 12 days and ~8 mm (Fig. 2A); the yolk and oil globule of these larvae were depleted within 4–5 days and ~5 mm. In the area of evagination, the foregut was sheathed by a mucosa composed of a single layer of simple cuboidal epithelial cells that was underlain by a thin, single-cell layer of circular smooth muscle,

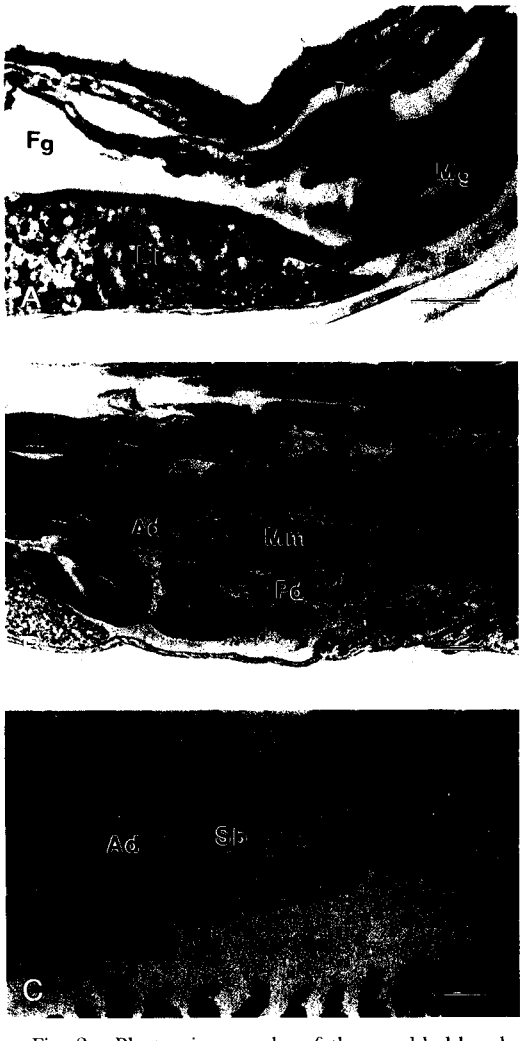

Fig. 2. Photomicrographs of the gas bladder development of *Brevoortia tyrannus* (hematoxylin-eosin). (A) Evagination (arrowhead) of the swimbladder from the foregut (Fg) above the posterior liver (Li) and anterior of the midgut (Mg) of a 15-day, 8-mm larva (scale bar = 50 μm). (B) Swimbladder with pneumatic duct (Pd) and anterior duct (Ad) and expanded muscularis mucosa (Mm) of a 33-day-old, 16-mm larva (scale bar = 100 μm). (C) Junction of the anterior duct and swimbladder (Sb) of a 22-day, 12-mm larva (scale bar = 100 μm).

a subjacent lamina propria of fibrous connective tissue, and a thin serosa. No definitive submucosa or muscularis was evident. At 16 days (~10 mm), the pneumatic duct formed from the proximal evagination, whereas the distal evagination was noticeably expanded into a swimbladder with a narrow lumen. A muscularis mucosa, composed of a single layer of circular smooth muscle that was continuous with the underlying muscle layer of the foregut, developed at this time. This muscle layer was so termed because of its close application to the mucosa and its lack of separation by a definable submucosa. Thereafter, the swimbladder continued to expand as the muscularis mucosa thickened (Fig. 2B). For the remainder of the larval period, before the onset of metamorphosis, the swimbladder had a tunica interna with a mucosa of simple cuboidal epithelial cells (~5 μm thick) subtended by areolar connective tissue (~5 μm), and a muscularis mucosa (~20 μm). The tunica externa had a layer of fibrous connective tissue (~10 μm) and a very thin serosa.

Three ducts enter or leave the swimbladder. The pneumatic duct originated at the caudodorsal wall of the foregut near the pyloric valve, inserted on the midmedial ventral wall of the swimbladder, and maintained the same tunicae as the swimbladder proper throughout larval development (Fig. 2B). At metamorphosis, during and after the development of a stomach, the pneumatic duct originated at the dorsal esophageal-gastric junction, just anterior of the pyloric valve. An anterior duct, originating at the medial craniodorsal swimbladder wall and inserting after diverticulation into paired precoelomic ducts near the pterotic bullae (Hoss and Blaxter, 1982), was evident within 20 days and ~12 mm (Fig. 2B). The mucosa of the pneumatic and anterior ducts developed rugae in larvae and maintained these folds through metamorphosis. A single layer of smooth, circular muscle composed the muscularis mucosae. Each duct was sheathed by a thin serosa.

The swimbladder assumed its adult form with metamorphosis of visceral organs beginning at about 40 days and ~18–19 mm, as a single-chambered organ. Two layers of smooth muscle, one circular and the other longitudinal, now composed the muscularis mucosa of the swimbladder, whereas a single layer of circular striated muscle composed the muscularis mucosa of the anterior duct. The anterior duct was funnel-shaped, and highly muscular, at its origin (Figs. 2C, 3A). Although the swimbladder lacks a rete mirabile, as do other clupeids, a bilateral, longitudinal slip of vascular tissue, heretofore undescribed, develops in the lateral wall of the anterior duct (Fig. 3A–B). The coelomic peritoneum above the swimbladder became pigmented with stellate melanocytes during evagination. The tunica interna became silvered with presumed guanine at metamorphosis, thus providing a barrier to the diffusion of gas (Lapennas and Schmidt-Nielsen, 1977).

Physiology: Brevoortia tyrannus.—Given larval behavior and swimbladder inflation observed in

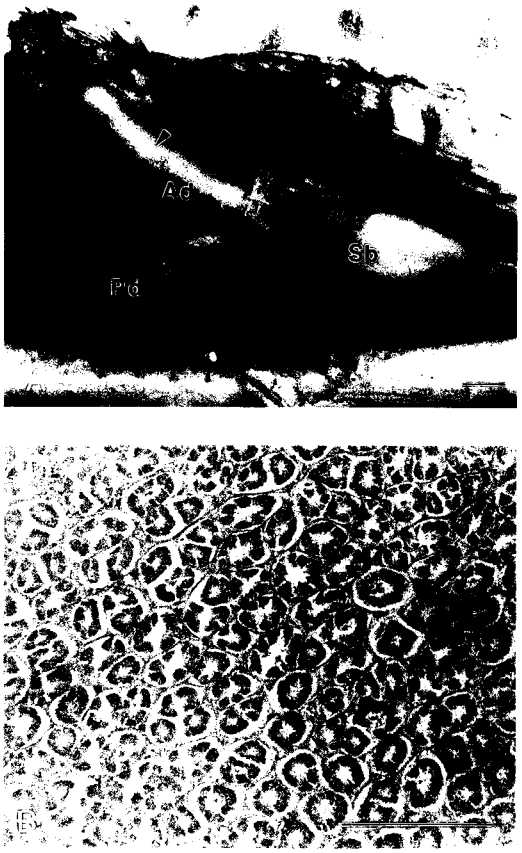

Fig. 3. The swimbladder, pneumatic duct, and anterior duct of an adult *Brevoortia tyrannus*, 195 cm (standard length). (A) Photograph showing the lateral slip of vascular tissue (arrowhead; scale bar = 1 cm). (B) Photomicrograph of the lateral slip of vascular tissue (hematoxylin-eosin; scale bar 30 μm).

the laboratory and in the sea, larger percentages of larvae with inflated swimbladders would be expected during the evening, after the replenishment of gas. In Onlsow Bay, swimbladders were inflated only in the early evening (Table 1). The greatest percentage of larvae with inflated swimbladders was at ~8 m when larvae were possibly descending after recent swimbladder reinflation.

Morphology: Leiostomus xanthurus.—Evagination of the swimbladder was first evident at two days and ~2.5 mm when larvae were nearing yolk and oil-globule depletion. Evagination was along the anterior incipient gut, above the yolk and oil globule, posterior of the coeliacomesenteric artery (Fig. 4A), and near the position of the developing liver and pancreas. In this area, the incipient gut and the evaginating swimbladder lacked an epithelial striated border of microvilli. It was sheathed by several layers of simple cuboidal mucosal epithelial cells (15 μm), with a subjacent layer of connective tissue (lamina propria) and a serosa. The cytoplasm of mucosal epithelial cells was eosinophilic. No submucosa or muscularis was identifiable. Three circulatory vessels lay caudoventral to the evaginating gas bladder (Fig. 4B). The larval swimbladder and pneumatic duct became fully formed at six days and ~3 mm as the incipient gut was intorting and becoming segmented. The swimbladder now had a lumen. A thin muscularis mucosae composed of circular smooth muscle was evident. Remnant stellate and interconnected mesenchymal cells were present in the dorsal coelom, from which this muscle derived. The pneumatic duct, which also has a thin muscularis mucosa, originated just anterior of the pyloric valve on the right lateral side and inserted at the caudoventral corner of the swimbladder.

For the remainder of the larval period, the tunica interna of the swimbladder had a mucosa of simple cuboidal epithelial cells, a lamina propria of areolar connective tissue, and a muscularis mucosa of smooth muscle (~10 μm); a serosa composed the tunica externa. The ventral and lateral mucosa was thickened and its cytoplasm became eosinophilic, vacuolar, and invested with granular, PAS-reaction products similar to those seen in the gas gland tissue during metamorphosis (Fig. 4C). Craniodorsal to this mucosa were three arterioles served by the coeliacomesenteric artery; caudoventral were three venules that drained into the profundal caudal vein (Fig. 5A). These circulatory vessels were not plexiform and constituted neither the presumptive rete mirabile nor the anlage of the spleen (Morrison, 1993), an organ that forms independently in the left lateral region of the liver after 20 days and ~3.5 mm.

The pneumatic duct atrophied after 20 days and had no discernable lumen after 40 days (~7 mm), the onset of metamorphosis. No transmission of gas through the pneumatic duct was evident after initial swimbladder inflation; there were no gas bole as there frequently are in *B. tyrannus*. A ventral mesenteric attachment to the alimentary canal is all that remained of the pneumatic duct thereafter.

The muscularis mucosa of the swimbladder atrophied as well with only a two muscle fiber layer remaining after 30 days (~5 mm). No muscle remained in the swimbladder wall after metamorphosis.

The swimbladder began to assume its adult form—a simple, single-chambered organ—at the onset of metamorphosis. Gas gland tissue,

TABLE 1. PERCENTAGE OF LARVAL *Brevoortia tyrannus* AND *Leiostomus xanthurus* WITH INFLATED SWIMBLADDERS, NUMBERS AT DEPTH, AND MEAN SWIMBLADDER VOLUMES (OBSERVERD AND ADJUSTED) FOR LARVAE COLLECTED IN THE ATLANTIC OCEAN OFF NORTH CAROLINA (NA = NOT APPLICABLE; ND = VOLUMES NOT DETERMINED BECAUSE OF POSSIBLE EXPULSION OF GAS THROUGH THE PNEUMATIC DUCT).

Species	Capture time (EST)	Median capture depth (m)	Percent captured at depth	Percent captured and inflated	Mean volume (SE) observed adjusted (μl)
B. tyrannus	1200	8	59	0	NA
		16	16	0	NA
		26	8	0	NA
	1700	8	33	0	NA
		15	10	0	NA
		28	0	0	NA
	1900	8	73	61	ND
		18	44	46	ND
		28	58	51	ND
L. xanthurus	1200	8	3	67	0.015780 0.008683 (0.005546) (0.003059)
		16	25	92	0.016827 0.006408 (0.002097) (0.007984)
		26	35	71	0.023293 0.006406 (0.002120) (0.000583)
	1700	8	2	100	0.014645 0.080556 (0.001056) (0.000581)
		15	14	71	0.009349 0.003703 (0.002568) (0.001017)
		28	18	6	0.007922 0.004357 (0.003125) (0.001719)
	1900	8	5	60	0.007922 0.004357 (0.003125) (0.001719)
		15	3	100	0.017579 0.006216 (0.001918) (0.000678)
		28	42	83	0.028437 0.007409 (0.002799) (0.000730)

first recognized in larvae, remained mucosal rather than submucosal. An organized, plexiform rete mirabile first appeared in larvae at metamorphosis (Fig. 5B). Both lay in the ventral wall of the swimbladder, the gland tissue as a craniad enveloping crescent of tissue, the rete as a medial ventral mass (Fig. 5B). The gas gland was bipolar (sensu Scholander, 1954) with arterioles and venules of the rete arranged in a checkerboard, not hexagonal, pattern (Fig. 5C). The dorsal and lateral mesentery that suspends the swimbladder became pigmented with melanocytes soon after evagination, but the dorsal coeliac peritoneum lacked pigmentation. Guanine was apparent in the tunica interna after metamorphosis.

Physiology: Leiostomus xanthurus.—Larvae initially inflate the swimbladder by forcing atmospheric gas into the swimbladder through the pneumatic duct. At the time of initial inflation, approximately seven days or 2.4 mm, about 50% of the swimbladders of living larvae within rearing tanks and with access to the atmosphere were inflated with gas; swimbladders not inflated were fluid-filled. Mortality in all three experimental vessels was high; no larvae remained alive after eight, nine, and 10 days in the one-, four-, and five-liter vessels. Swimbladders of larvae deprived access to the atmosphere formed but remained fluid-filled. When deprived access, larvae congregated at the barrier interface. Some larvae as old as 25 days and ~4 mm were seen with fluid-filled swimbladders in the laboratory and in the sea.

Larvae are capable of secreting gas into the swimbladder shortly after initial swimbladder inflation and improve this capacity as they grow; larvae restored gas volume following a pressure increase and consequent gas-volume decrease. If temperature is held constant, a pressure increase of 101 kPa will reduce the volume of gas in a compliant swimbladder by one-half. All neutrally buoyant young larvae at ambient pres-

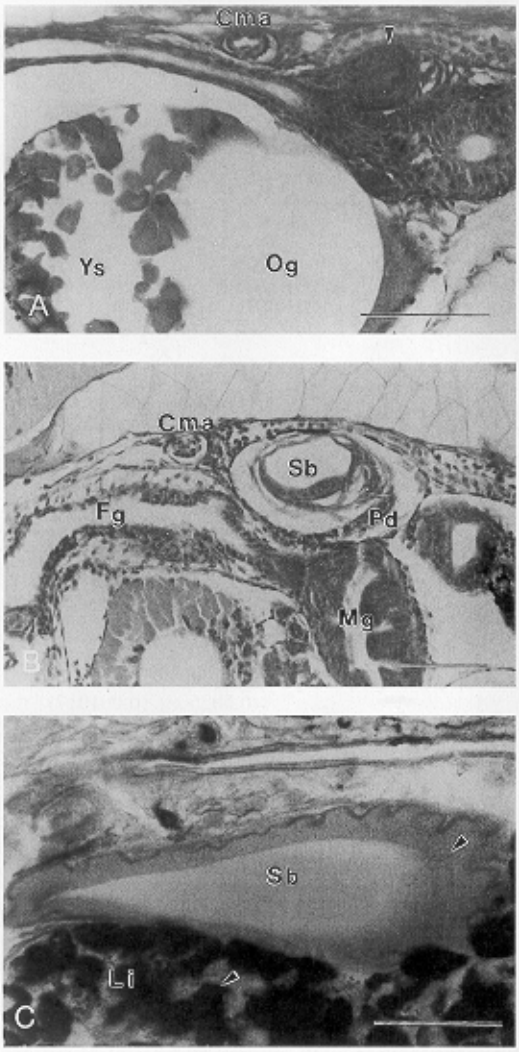

Fig. 4. Photomicrographs of swimbladder development of *Leiostomus xanthurus* (hematoxylin-eosin). (A) Evagination (arrowhead) of the swimbladder from the incipient gut dorsal to the yolk sac (Ys) and oil globule (Og) and caudal to the coelliacomesenteric artery (Cma) of a two-day-old, 2-mm larva (scale bar = 50 μm). (B) Swimbladder dorsad of the fore- and midgut and pneumatic duct of a five-day, 2.5-mm larva (scale bar = 50 μm). (C) Mucosal epithelium of the swimbladder and liver hepatocytes of a 21-day-old, 4-mm larva with small granular, glycogen deposits (arrowhead) in epithelium and large glycogen deposits in hepatocytes (PAS-hematoxylin; scale bar = 50 μm).

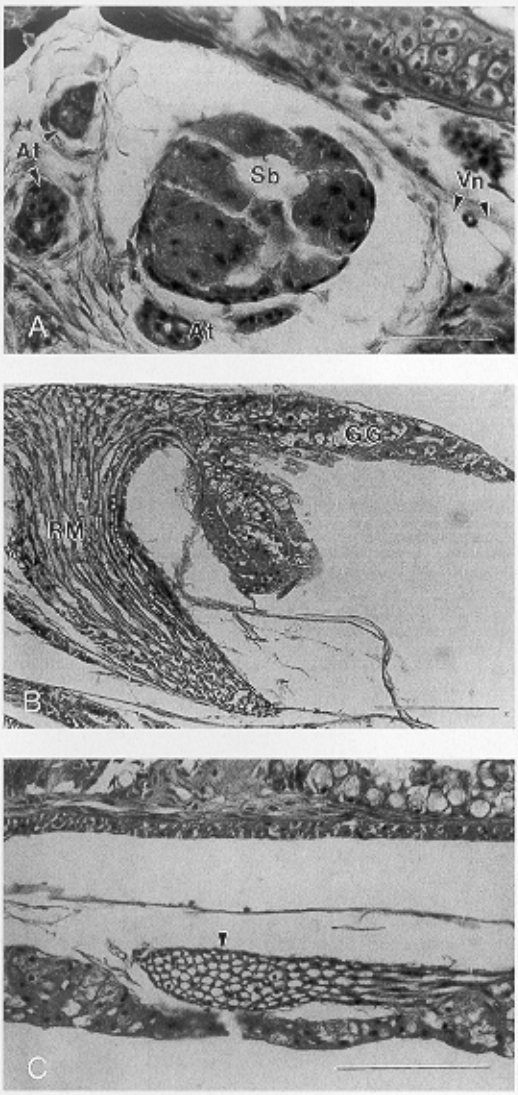

Fig. 5. Photomicrographs of the circulatory vessels that support the swimbladder of *Leiostomus xanthurus* (hematoxylin-eosin). (A) Arterioles (At) and venules (Vn) of a 24-day-old, 3.5-mm larva (scale bar = 50 μm). (B) rete mirabile (RM) and gas gland (GG) tissue of a 13-mm larva (scale bar = 50 μm). (C) Checker-board arrangement of rete vessels (arrowhead) of a 13-mm SL larva (scale bar = 50 μm).

sure rapidly descended to the bottom of the chamber upon application of 101 kPa of pressure above ambient (Fig. 1); older larvae became negatively buoyant and sank but checked their descent by swimming upward. Within one to seven hours, about half of surviving larvae of all ages over three days were again suspended, motionless, in the water of the hyperbaric chamber, which signified that these larvae had recovered neutral buoyancy by restoring gas volume. With temperature held constant, pressure release will result in a doubling of the restored gas volume. Upon pressure release, surviving larvae rapidly ascended to the water-oil interface and had swimbladders that were distended caudally; other larvae were on the bottom of the

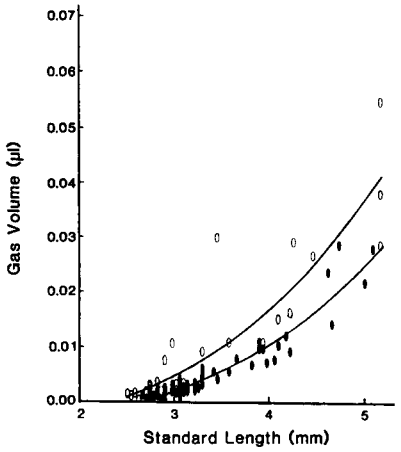

Fig. 6. The volume of gas in the swimbladder and notochord or standard lengths of larval *Leiostomus xanthurus*: closed symbols represent volumes of larvae measured before a 7-h application of 101 kPa pressure above ambient; open symbols, after application of pressure

hyperbaric chamber and were moribund or dead, with little or no gas in the swimbladder. Little gas in these larvae signifies that gas diffused rapidly through the swimbladder wall. The observed position of larvae within the hyperbaric chamber before, during, and after the application of pressure indicates that a pressure increase of 101 kPa, commensurate with a 10-m descent in the sea, halves the gas volume of a compliant swimbladder, but within 1–7 h, surviving larvae had restored this volume and regained neutral buoyancy.

Gas secretion into (and diffusion from) the swimbladder is a function of the surface area of the adult swimbladder (Lapennas and Schmidt-Nielsen, 1977); the capacity to secrete gas increases curvilinearly with the length of larvae (Fig. 6). The relation of measured gas volume to the cube of length was rectilinear (Table 2). The slopes of the regressions of gas volume and the cube of length, before and after the application of pressure, diverged. Slopes of these regressions differed significantly [analysis of covariance (ANCOVA); $F = 13.25$, df = 1,90; $P < 0.005$].

Some of the measured gas volumes after the application of pressure were approximately double that of initial volumes; many were not. Comparison of estimates predicted from regressions of volume and the cube of length, before and after the application of pressure (Table 2), indicates a 32.9% increase in gas volume. Diffusion of gas from the swimbladder, while larvae were being recovered from hyperbaric chambers and measured (individually), probably accounts for this difference and variation.

The rate of gas secretion increased with the length of larvae. Some, but not all, older and longer larvae achieved neutral buoyancy within one hour after the application of pressure. Younger and shorter larvae required seven hours. Calculated as an overall rate, gas secretion ranged from 3.7 to 5.5 $\times 10^{-4}$ µl · h^{-1} for 11-day larvae. This rate ranged from 1.4 to 8.4 $\times 10^{-3}$ µl · h^{-1} for 24-day larvae.

Given the previously observed vertical distribution of larvae in the sea, larvae would be expected to have inflated swimbladders over a 24-h period. In Onlsow Bay, most larvae had inflated swimbladders throughout the day and at night at all depths (Table 1). In the main, the vertical distribution of larvae observed in the present work was centered at mid- and deep depths.

Given the capability of adjusting swimbladder gas volume to achieve neutral buoyancy in the laboratory, larvae would be expected to have equivalent volumes at all depths in situ throughout most of their vertical depth range. Time had no significant effect on observed gas volumes measured at the surface aboard ship (multiple regression; $P > 0.0905$), whereas depth of capture ($P < 0.001$) and the cube of length ($P < 0.001$) did (temperature was not considered because the water column was isothermal at approximately 15 C). Overall, in situ gas volumes, adjusted for temperature and pressure change from depth where they were captured and the surface where they were measured, overlapped (Table 1). Adjusted volumes were not significantly different among depths, after the effect of larval length was removed (by using the common slope of the combined volumes (all times

TABLE 2. RELATIONS OF THE VOLUME OF GAS IN THE SWIMBLADDER AND THE CUBE POWER OF NOTOCHORD OR STANDARD LENGTH OF LARVAL *Leiostomus xanthurus* BEFORE AND AFTER THE APPLICATION OF 101 kPa PRESSURE ABOVE AMBIENT.

	Intercept	(SE)	Slope	(SL)	R^2
Before pressure	−0.004451	(0.200471)	0.000235	(0.000010)	0.92
After pressure	−0.004132	(0.004132)	0.000329	(0.000026)	0.82

and depths); ANCOVA per Peters (1983) and Packard and Boardman (1988), df = 2, 98; $P = 0.4762$). Equivalent gas volumes at depth indicate that larvae were adjusting gas volume against loss to diffusion, to maintain neutral buoyancy.

Discussion

Morphology.—Development of the swimbladder through simple evagination of the foregut of *B. tyrannus* and *L. xanthurus* requires no major renovation of tissue or hitherto unreported movement of germ cells. The lining of the swimbladder, simple cuboidal epithelium, is in place in the foregut of *B. tyrannus* and anterior incipient gut of *L. xanthurus*. The closely applied, underlying muscle is in place in larval *B. tyrannus* and is added, from mesenchyme, in larval *L. xanthurus*. This condition might not exist for other species reported to form the swimbladder from an evagination of other segments of the developing alimentary canal (e.g., Harden Jones and Marshall, 1953; Schartz, 1971; Boulic and Gabaudan, 1992), inasmuch as posterior segments of the embryonic and larval alimentary canal (the mid- and hindgut) are typically lined with columnar epithelial cells with an apical striated border (Govoni et al., 1986). Descriptions of swimbladder development have used inappropriate nomenclature to describe embryonic or larval alimentary canal segmentation.

In fishes that spawn large, demersal or adhesive, and slowly developing eggs that hatch precocial larvae (sensu Balon, 1979), the swimbladder develops embryonically, or within yolk sac larvae, whereas, in fishes that spawn small, planktonic eggs that hatch altricial larvae (Balon, 1979), the swimbladder typically develops in free-feeding larvae. Evagination at the end of yolk sac and oil-globule depletion in larval *L. xanthurus* (Fig. 7), when the gut is in transition from an unsegmented incipient gut to a segmented and differentiated larval alimentary canal, is common among species with small, planktonic eggs. Evagination of the swimbladder from the larval foregut long after yolk and oil-globule depletion and gut segmentation as in *B. tyrannus* (Fig. 7) is less common but conforms with swimbladder development in the engraulid *Engraulis mordax* and other clupeiforms (O'Connell, 1981). An organized rete mirabile is reported to develop in embryonic *Lepomis macrochirus* (Duwe, 1952) and *Opsanus tau* (Tracy, 1959) and is evident shortly after yolk depletion in *Melanogrammus aeglefinus* (Schwarz, 1971), *Gadus morhua* (Morrison, 1993), *Percina caproides*

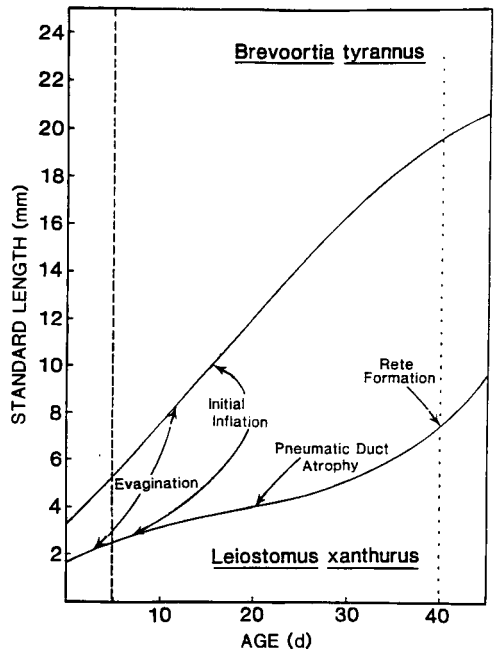

Fig. 7. Schematic representation of the feature events of swimbladder ontogeny in *Brevoortia tyrannus* and *Leiostomus xanthurus*. Growth is described for *B. tyrannus* from Powell (1994) before yolk and oil-globule depletion and from Warlen (1992) thereafter. For *L. xanthurus*, growth is described from Powell and Gordy (1980) before yolk and oil-globule depletion and from Flores-Coto and Warlen (1993) thereafter (the vertical broken line denotes yolk and oil-globule depletion; the dotted line, the onset of metamorphosis).

(Grizzle and Curd, 1978), *Latris lineata* (Goodsell et al., 1996), *Pagrus major* (Yamashita, 1982), and *Morone saxatilis* (Bulak and Heidinger, 1980). A rete does not develop until the onset of metamorphosis in *L. xanthurus*, *Micropterus salmoides* (Johnston, 1953), and *Solea solea* (Boulic and Gabaudan, 1992).

Other details of swimbladder development differ between *B. tyrannus* and *L. xanthurus* and from the typical teleostean pattern. The muscularis mucosa of the swimbladder and pneumatic duct is thicker in larval *B. tyrannus* than it is in larval *L. xanthurus*, wherein it disappears by metamorphosis. Muscularis mucosa in the larvae of both species probably functions by drawing gas into, and forcing gas from, the swimbladder, initially in *L. xanthurus*, and diurnally throughout larval life in *B. tyrannus*. This muscle layer also forces gas to and from the otic bullae in *B. tyrannus* (Hoss and Blaxter, 1982). No intrinsic striated muscle (of voluntary neural control) was found in the muscularis mucosae of the swimbladder or the pneumatic duct of *L.*

xanthurus, nor is striated muscle typically reported in these organs of most adult fishes (Harden Jones and Marshall, 1953; Groman, 1982). Striated muscle was found in the anterior duct of *B. tyrannus;* it is reported in the pneumatic duct of *Ictalurus punctatus*, a physostomous fish (Grizzle and Rogers, 1976), and the swimbladder of Triglidae (*Bellator* spp. and *Prionotus* spp.) and *O. tau*, physoclistous fishes (Tracy, 1959; Evans, 1973).

The morphology of the juvenile and adult swimbladder of *B. tyrannus* and *L. xanthurus*, as described here, differs in some details from earlier descriptions. Whitehead and Blaxter (1989) described a small anal duct that connects the caudoventral swimbladder to an anal pore, and Forward et al. (1994) reported gas expulsion through the anus, but Tracy (1920) describes no anal communication with the swimbladder, and its development was not recognized in the specimens examined. The swimbladder of *B. tyrannus* lacks a rete mirabile, as do other clupeids, but the vascular tissue of the anterior duct resembles the circumscribing "vascularis" of the anterior swimbladder of *Sardinops caerulea* (O'Connell, 1955).

Physiology.—The mechanism for initial swimbladder inflation remains in question for many fishes. In physostomous fishes, *B. tyrannus* among them, the swimbladder is inflated initially with atmospheric air forced through the gut and pneumatic duct (e.g., Hoar, 1937; Uotani, 1973; Allen et al., 1976). Most physoclistous fishes inflate the swimbladder by this means as well (e.g., Battaglene et al., 1994; Rieger and Summerfelt, 1998; Goolish and Okutake, 1999), although some appear to inflate the swimbladder with gas generated internally (McEwen, 1940; Johnston, 1953; Doroshev et al., 1981). With either mechanism, failure to inflate causes suspension of swimbladder development (e.g., Chatain, 1986), disease, and death (e.g., Marty et al., 1995). In physoclistous *L. xanthurus*, the swimbladder inflates initially with atmospheric gas and secretes gas internally soon thereafter.

Larval *L. xanthurus* are remarkably capable of accomplishing gas secretion against appreciable, but realistically encountered, pressure, and without benefit of either a rete mirabile or abundant erythrocytes (JJG, unpubl.). Secretion of gas into the teleostean swimbladder is typically a two-staged process. The process entails: (1) the reduction of nitrogen and carbon dioxide solubility, and oxygen affinity and cooperativity of hemoglobin, in the blood by acidification; and (2) the multiplication of partial pressures by counter current exchange in the rete mirabile (Pelster and Scheid, 1992; Pelster, 1995). An organized rete mirabile is not recognizable in *L. xanthurus* until metamorphosis. Doroshev et al. (1981) argue that the tissue of the larval mucosal epithelium is a transient functional specialization. This glandular tissue is characteristically vacuolar (McEwen, 1940; Johnston, 1953; Goodsell et al., 1996), eosinophilic (Doroshev et al., 1981), and invested with glycogen granules (Marty et al., 1995). Eosinophilia probably results from the abundant mitochondria of these metabolically active cells (Morrison, 1993) and the rich investment of glycolytic enzymes (Morris and Albright, 1984; Pelster and Scheid, 1991). Glycolysis and the resulting lactate and CO_2 production drives the salting-out of gases dissolved in the blood, principally CO_2 and N_2, as well as forcing decreased oxygen affinity and cooperativity of hemoglobin in erythrocytes, the Root effect (Pelster and Scheid, 1992). The thickened, eosinophilic, vacuolar, and PAS-reactive mucosal epithelium within the ventral gas bladder wall of larval *L. xanthurus* probably functions in altering the ionic composition and pH of circulating fluids. In larval *Danio rerio*, swimbladder inflation is impaired and gas volume reduced by ablation of hemoglobin (Pelster and Burggren, 1996), but there are few circulating erythrocytes and little detectable hemoglobin in larval *L. xanthurus* (JJG, unpubl.).

Larval fishes are developing organisms; as such their visceral organ systems may not acquire full functional capacity until metamorphosis or may even function differently for larvae than they do for juvenile or adult fishes. *Brevoortia tyrannus* and *L. xanthurus* exhibit differences in swimbladder development, but in both species the swimbladder functions to provide buoyancy compensation and this function explains, in part, their depth distribution in the sea, where they reside together. The swimbladder develops late in *B. tyrannus*, well after yolk and oil-globule depletion (Fig. 7) at a time when the specific gravity (in seawater) of larvae is increasing rapidly (Power and Walsh, 1992). Larval *B. tyrannus* inflate the swimbladder through the pneumatic duct and modulate the volume of gas with a diel periodicity for the balance of larval life (Hoss and Phonlor, 1984; Hoss et al., 1989; Forward et al., 1999). By ascending to the surface each evening and sinking slowly as gas diffuses, or is expelled, older larval *B. tyrannus* reside, on average, in the upper regions of water overlying the continental shelf (J. A. Hare and J. J. Govoni, unpubl.). The swimbladder of *L. xanthurus* develops early,

soon after yolk and oil globule-depletion; the pneumatic duct atrophies in older larvae. Larval *L. xanthurus*, lacking the adult complement of gas secreting organs and tissues, modulate gas volume by internal gas secretion and achieve neutral buoyancy, as do most adult physoclistous fishes that are neutrally buoyant at all but the deepest extremes of their depth range (Alexander, 1972). Larval *L. xanthurus* reside, on average, at mid- and deep depths but range ~10 m about this average over a diel period. The rate of gas secretion can meet the demand for neutral buoyancy that this oscillation in depth requires. The larvae of physoclistous fishes other than *L. xanthurus* are capable of buoyancy compensation, though not necessarily the achievement of neutral buoyancy (Yin and Blaxter, 1987; Kitajima et al., 1993), but the mechanisms of inflation are not well understood.

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